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TREATMENT FOR DIABETES

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(57) Claim

1. A method of treatment of a mammalian patient suffering from diabetes which includes the transplantation into the patient of viable porcine islets capable of producing porcine insulin within its host, said islets having been extracted from a piglet at or near full term gestation,

wherein said islets were treated during preparative procedures with nicotinamide and/or any compound exhibiting similar growth promoting and cytoprotective effects,

and wherein said patient, at least for a period after such transplantation, is administered nicotinamide and/or a compound exhibiting similar growth promoting and cytoprotective effects.

14. A preparation capable of being injected into a mammalian patient to provide transplantation, including an effective amount of porcine islets capable of producing porcine insulin, said islets having been extracted from a piglet at or near full term gestation, said islets having been treated during preparative procedures with nicotinamide and/or any compound exhibiting similar growth promoting and cytoprotective effects.

ABSTRACT

The present invention relates to a method of treatment of a mammalian patient suffering from diabetes (including humans) which involves the transplantation into the mammal of viable porcine islets capable of producing insulin within its host.

AUSTRALIA

PATENTS ACT 1990

PATENT REQUEST FOR STANDARD PATENT

We, DIATRANZ LIMITED, being the person identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying standard complete specification.

Full application details follow:

Applicant:	DIATRANZ LIMITED
Address:	60 Parnell Road, Parnell, Auckland, New Zealand
Nominated Person:	As above
Address:	As above
Invention Title:	"TREATMENT FOR DIABETES"
Name of actual Inventor:	Robert Bartlett ELLIOTT

ASSOCIATED PROVISIONAL APPLICATION DETAILS:

Application Number: PO 8780 dated 26th August, 1997

Drawing number recommended to accompany the abstract: None

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DATED this 25th Day of August, 1998.
DIATRANZ LIMITED

by
Fellow Institute of Patent Attorneys of Australia
of BALDWIN SHELSTON WATERS

To: The Commissioner of Patents
WODEN ACT 2606

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AUSTRALIA

PATENTS ACT 1990

COMPLETE SPECIFICATION

FOR A STANDARD PATENT

ORIGINAL

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Invention Title:	"TREATMENT FOR DIABETES"

Details of Associated Provisional Application No. PO 8780 dated 26th August, 1997

The following statement is a full description of this invention, including the best method of performing it known to us:-

TECHNICAL FIELD

The present invention relates to improvements in and/or relating to the treatment of diabetes.

BACKGROUND

It has been about 100 years since the first attempt to transplant viable insulin producing tissue - in this case from a dog, into diabetic humans. Islet cell allotransplants were first attempted in the late 1970s, and efforts continue to be made up to this time. Islets from mid-trimester foetuses have been uniformly unsuccessful. Only in the 1980s did the progress of two decades of basic scientific research result in a better technique to purify a high yield of human islets.

Using closely H.L.A. matched adult donors, with great care to ensure viability, purity and adequate numbers of islets (> 200,000) some limited success has been attained in reversing diabetes (about 30% success at 1 year of follow up. In all of these attempts, the recipient has received continuous immunosuppression (which has itself presented unwanted dangers to the recipient) usually including cyclosporine. More recently, close attention to diabetes control following transplantation has been added to the protocols.

Allo-transplantation of islets into diabetic subjects has, in addition to the usual problems of vascularization and rejection common to any such transplant, the additional problem of recurrence of 'insulitis' and b cell destruction inherent to diabetes - as exemplified by the short term success, but longer term failure of pancreatic segmental transplants between identical twins discordant for diabetes. (See *SUTHERLAND D E R, MATAS A J, GOETZ F C, NAJARIAN J S. Transplantation of dispersed pancreatic islet tissue in humans: autografts and allografts. Diabetes 1980; 29 (Suppl.1): 31-44).*

Whilst the ideal transplant donor tissue should be H.L.A. identical with the recipient, this is the combination most likely to result in disease recurrence in the graft. Transplantation of mice rendered diabetic with streptozotocin with donor mouse islets different in several major H.L.A. loci has been carried out successfully, using purification technique to eliminate non-islet cell contaminants. (See *BOWEN K M, ANDRUS L, LAFFERTY K. Successful allotransplantation of mouse pancreatic islets to non-immunosuppressed recipients. Diabetes 1980: 29:98).*

These transplants were carried out without any form of recipient

immunosuppression. However, when similar transplants were made into spontaneously diabetic (NOD) mice, they were unsuccessful, unless the recipients were treated with nicotinamide and desferrioxamine, (*See NAMIKOS I N. PROWSE S J. CAROTENUTO P, LAFFERTY K J. Combined treatment with nicotinamide and desferrioxamine prevents islet allograft destruction in NOD MICE. DIABETES 1986; 35: 1302*) which appeared to prevent disease recurrence in the transplanted tissue. Nicotinamide can prevent diabetes in this strain of mouse (*See YAMADA K, NONAKA K, HANAFUSA T, MIYAZAKI A, TOYOSHIMA H, TARUI S. Preventative and therapeutic effects of large dose nicotinamide injections on diabetes associated with insulitis, Diabetes 1982; 31: 749-753*) although its precise mode of action is subject to much debate. Desferrioxamine is thought to act as repressor of free-radical generation.

Xenotransplantation (pig to streptozotocin induced diabetic mouse) of islets has previously only been successfully carried out in athymic nude mice (*See KORSGREN O, JANSSON L, EIZINK D, ANDERSON A. Functional and morphological differentiation of fetal porcine islet like cell clusters after transplantation into nude mice. Diabetologia Vol 34: 379-386, 1991.*)

These mice lack T cells, but are able to generate antibodies to certain antigens via nonthymic dependent B cells. Whilst xenotransplantation may be the best option to prevent disease recurrence grafts made into spontaneously diabetic animals or humans, because of great dissimilarities in tissue antigens, the likelihood of rejection is correspondingly increased.

Pig islets have been prepared and injected into the portal vein of a diabetic human subject - with only transient evidence of production of pig (pro)insulin (*See KORSGREN O, GROTH C G, ANDERSON A, HELLERSTRON C, TIBELL A, TOLLEMAR J, BOLINDER J, OSTMAN J, KUMAGAI M, MOLLER E, BJOERSDORFF A. Transplantation or Porcine fetal pancreas to a Diabetic Patient. Transplantation Proceedings vol 24, No. 1 (February), 1992: 352-353*) xenotransplantation of other organs (spleen, liver has been notably unsuccessful despite treatment with 'state of the art' immunosuppressants (e.g. the recent Pittsburgh experiences). On the other hand, these organs cannot be rid of lymphocytes and other active antigen presenting cells.

It is an object of the present invention to overcome or at least ameliorate one or more of the disadvantages of the prior art or at least provide a useful alternative thereto.

SUMMARY OF THE INVENTION

The present invention relates to a method of treatment of a mammalian patient suffering from diabetes (including humans) which involves the transplantation into the mammal of viable porcine islets capable of producing insulin within its host. Such transplantation to date has not been sufficiently successful without side effects associated with concomitant continuous immunosuppression.

The present invention also includes preparations useful for such a method of treatment.

In a first aspect the present invention consists in a method of treatment of a mammalian patient suffering from diabetes which includes the transplantation into the patient of viable porcine islets capable of producing porcine insulin within its host, said islets having been extracted from a piglet at or near full term gestation,

wherein said islets were treated during preparative procedures with nicotinamide and/or any compound exhibiting similar growth promoting and cytoprotective effects,

and wherein said patient, at least for a period after such transplantation, is administered nicotinamide and/or a compound exhibiting similar growth promoting and cytoprotective effects.

Preferably, the mammalian patient is a human patient.

Preferably the administration of nicotinamide and/or a compound exhibiting analogous effects is administered to the mammal along with a source of protein that substitutes for bovine protein including casein.

Preferably said piglet from which the islets have been extracted is newborn.

Preferably the preparation is substantially as hereinafter described and may include a cryogenic storage period prior to thawing and transplantation.

In a further aspect the present invention consists in a preparation capable of being injected into a mammalian patient to provide transplantation of a type referred to in the method of the present invention, said preparation having a viable insulin producing quantity of islets that have been extracted from a newborn piglet into nicotinamide and/or a compound exhibiting analogous effects.

In a further aspect the present invention consists in the said preparation in a cryogenically stored form.

In still a further aspect the present invention consists in a transplantable quantity

of a preparation in accordance with the present invention having at least 100,000 porcine islets that have been and/or are in a nicotinamide containing environment and which on transplantation are able to multiply.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

We have succeeded with allotransplatation into diabetic NOD mice using neonatal donor tissue islets from the pig.

A novel feature has been the use of neonatal donor tissue as

i) islets are easier to prepare in partially purified form from very young animals than older animals

ii) the islets are still capable of some replication, compared with adult islets, and the use of nicotinamide in the culture media used in islet preparation.

Islets flourish in media enriched with 10m2 nicotinamide. Cell numbers, D.N.A. content and insulin production capacity are enhanced. (*See SANDLER S, ANDERSON A. Long term effects of exposure of pancreatic islets to nicotinamide in vitro on DNA synthesis, metabolism and Beta cell function. Diabetologia 1986; 29-199).*

The replication and maturation of foetal islets is improved by such treatment *(See SANDLER S, ANDERSON A. Stimulation of cell replication in transplanted pancreatic islets by nicotinamide treatment. Transplantation 1988; 46(1):30-31).*

Cytokines which induce MHC proteins also have b cell cytotoxic effects which are prevented by nicotinamide *(See MANDRUP-POULSEN T, BENDTZEN K, NIELSEN J, BENDIXEN G, NERUP J. Cytokines cause functional and structural damage to isolated islets of Langerhans. Allergy 1985, 40: 424-429 and KOLB H, BURKART U, APPELS B, HANNENBERG, KANTWERK-FUNKE G, KIESEL U, FUNDA J, SCHRAERMEYEN U, KOLB-BACHOFEN V. (1990). Essential contribution of macrophages to islet cell destruction in vivo and in vitro. J Autoimmun 3: 1-4).*

Nicotinamide pretreatment suppresses Class 2 M.H.C. expression on β cells. *(See YAMADA K, MIYAJIMA E, NONAKA KYOHEI. Inhibition of cytokine-induced MHC class II but not class I. Molecule expression on mouse islet cells by Nicotinamide and 3 Aminobenzamide. Diabetes vol. 39: September 1990: 1125-1130).*

Without being tied to a theory we believe nicotinamide may therefore prevent antigen presentation by β cells during the traumatic process of purification of islets from other pancreatic components, as well as producing more and more biologically active β

cells. We believe also that other compounds may exhibit a similar activity provided any such compound has functional homology with nicotinamide.

Nicotinamide alone can prevent diabetes in this strain if given early enough, *(See REDDY S, BIBBY N, ELLIOTT R. Dietary prevention and enhancement of diabetes in the NOD mouse. Lessons from Animal Diabetes II, Third International Workshop, March 1990: p 34)*. as can an 'elemental' or cow protein free diet *(See ELLIOTT R, REDDY S, BIBBY N, KIDA K. Dietary prevention of diabetes in the non-obese diabetic mouse. Diabetologia 1988, 31: 62-64)*.

Neither procedure alone is sufficient to prevent disease if given near to the time when diabetes usually occurs, but given together is effective *(See BIBBY N, ELLIOTT R B. Prevention of Diabetes in the NOD mouse with nicotinamide and Prosobee - Dosage and Timing are important. Abstract S. 60. Diabetes Research and Clinical Practice. Vol 14. Suppl 1, 1991)*.

We have found that in addition to continuance of the putative effects of nicotinamide used during the preparative procedures, an additional 'antidiabetic' effect can be obtained. This can be further enhanced by the elemental or soy protein diet.

This invention has established that purified newborn piglet islets, treated with nicotinamide during the preparative procedures, can be successfully transplanted into spontaneously diabetic mice treated with nicotinamide and a cow protein free diet (Table 1).

Without the special preparation of donor tissue, and treatment of the recipient, such transplantation is unsuccessful. Such transplantation is also unsuccessful in normal mice which have been rendered diabetic by the injection of a drug (streptozotocin) which poisons the insulin producing cells.

We have successfully carried out piglet islet transplants into mice which are born without a functional immune system and have been rendered diabetic with the drug. These immunodeficient mice did not develop any infections, confirming the sterility of the islet preparations.

From such experiments it can be concluded that xenotransplantation of islets (piglet to mouse) can be successfully carried out under the following conditions.

(i) islets are purified under aseptic conditions, in the presence of nicotinamide, and can be shown to produce insulin in response to glucose, before and after cryo-

preservation. The amount needed for successful transplantation in mice is about 100-200,000 islet cells.

(ii) the recipient mouse is -

(a) either spontaneously diabetic (NOD strain) or lacks a functional immune system.

(b) receives both nicotinamide from at least the time transplantation and preferably also a cow protein free diet from at least the time of transplantation.

Preferred embodiments of the invention will now be described with reference to the non-limiting examples.

Example 1: Preparation of newborn piglet islets

A litter of piglets are delivered by Caesarian section and their pancreases removed under sterile surgical conditions. The pancreases are diced, and incubated with collagenase under sterile conditions. The islets are then partially purified on a density gradient, and then explanted into tissue culture containing 10 m molar nicotinamide, for 1 week. At the end of this time, further purification has occurred. The islet cell are then checked for viability (dye inclusion) and ability to make insulin in vitro, in response to glucose. The cells and culture medium are checked for a battery of human and pig pathogens then cryopreserved. A small batch is thawed, rechecked for viability, insulin production in vitro, sterility, and in vivo ability to reverse diabetes and in vivo sterility. This procedure ensured that islets stored in liquid nitrogen will be viable, and sterile when thawed prior to transplantation.

Example 2: Islet cell allotransplants in non-obese diabetic mice (NOD)

The effects of the abovementioned procedures on allotransplantation into NOD mice of BALBC islets (given I.P.) is shown in Table 1.

TABLE 1

Diabetic NOD mice	# NOD transplanted	Permanent remission		Temporary remission		Total no. remission	
		#	%	#	%	#	%
Transplant Only	20	3	15	2	10	5	25
Transplant/ Nicotinamide	20	6	30	2	10	8	40
Nicotinamide Only	20	1	5	4	40	5	25

Fisher exact Probability Test was used and the difference between the three groups was statistically significant (0.0415).

The analysis of survival based on a proportional hazard model (PHREG) was used to assess whether or not post transplant survival was longer than expected with the general conclusion of a statistically significant benefit of transplantation compared with the survival experience of non transplanted mice (Chi square .0009)

The survival analysis of the combined treatment compared with control group shows a statistically significant benefit of this treatment (Chi square .0394).

We have shown 30% permanent cure of diabetes using newborn islet cells which have been cultured for 7 days with Nicotinamide, cryopreserved and transplanted (I.P.) into NOD mice given nicotinamide in drinking water.

Example 3: Porcine islet cell zenotransplants in non-obese diabetic (nod) mice.

Neonatal piglet islets were similarly prepared and injected into diabetic NOD mice (Table 2).

TABLE 2

Diabetic NOD mice	# NOD Mice transplanted	Permanent remission		Temporary remission		Total no. remission	
		#	%	#	%	#	%
Casein free diet only	20	2	10	3	15	5	25
Nicotinamide + casein free diet	20	7	35	6	30	13	65
Nicotinamide	16	1	6.3	2	12.5	13	18.8
Transplantation Only	13	2	15.3	4	30.8	6	46.2

The effect of the treatments on the diabetic status was investigated using a logistic regression model. Mice who had transplants were classified as having no remission, temporary remission or permanent remission using the number of aglycosuric days after treatment.

There was a significant difference between the treatments (p=.0023) the nicotiamide status was found to be statistically significant (p=.0076).

60% of the mice receiving nicotinamide and casein free diet reversed diabetes (12/20), six of them permanently, with a significant benefit from this trial compared with the other three groups.

In summary, allo. and xeno transplantation of islets into diabetic NOD mice can be successfully carried out under the conditions of the present invention..

We have successfully replicated the xenotransplantation of athymic nude mice. *(See KORSGREN O. JANSSON L. EIZIRIK D AND ANDERSON A. Functional morphological differentiation of fetal porcine islet like cell clusters after transplantation into nude mice. Diabetologia (1991) 34: 379-386)* but have been unsuccessful with the procedure in another immuno deficient strain (severe combined immunodeficient-SCID mice). These mice lack effective T & B cells, but do have active natural killer (NK) cells.

Example 4: Porcine islet cell transplantation into diabetic swiss mice (st2) 1992

We have also been less successful in attempts to xenotransplant Swiss mice (the non-diabetic progenitors of the NOD mouse) rendered diabetic with streptozotocin, using the same procedures which were successful in the NOD (Table 3).

TABLE 3

	# Swiss Transplanted	Permanent Remission #	Temporary Remission #	Total Remission #
Nicotinamide + Casein Free Diet	9	1	3	4
Transplant Only	6	1	1	2

It appears that the NOD mouse behaves immunologically more like the nude mouse, than the SCID or Swiss mouse. Lazarus et al. have demonstrated 'thymic anergy' in the NOD mouse over the age of 7 weeks. (See ZIPRIS D, LABARUS A, CROW A, HADZIJA M, DELOVITCH T. Defective thymic T cell activation by concanavalin A and Anti CD3 in autoimmune non-obese diabetic mice. The Journal of Immunology Vol. 146:3763-3771). This 'anergy' results in T-cells not being effectively 'trained' in the thymus, and thymic lymphocytes being unresponsive to Con A (Concanavalin A) and anti CD3. This defect is due to a genetically determined thymus-dependent phenomenon expressed in NOD mice.

Some credence can be given to the idea that the diabetic NOD mouse may be partially immunodeficient.

Diabetes in the human is similar to the disease in the NOD mouse, and may

respond to similar xenotransplantation procedures. The similarities and dissimilarities are listed below.

TABLE 4

	NOD MOUSE	HUMAN
Insulin and age dependent	+	+
Female > male	++	±
Insulitis, b cell destruction	+*	+
'HLA' association	+	++
Associated endocrine immuno pathology	+	+
MHC Class 2 non-aspartate (β chain 57) association	+	+
Islet cell antibodies	+	+
Insulin autoantibodies	+	+
Ie deficiency	+	?
'Thymic anergy'	+	?
Incidence	120-300 days (post-pubertal)	1-80 years (peripubertal predominantly)

* *initially peri-insular*

Example 5: The treatment of human patients

Full blood count, liver function tests, blood urea, nitrogen and creatinine were measured. A pregnancy test was also done where relevant.

Normal insulin treatment was continued up to 24 hours before transplantation when 4 hourly short acting insulin was prescribed according to blood glucose tests. The last insulin injection was given 4 hours before the transplant.

A cow's milk (c.m.) free diet commenced 1 week before transplantation and nicotinamide 1.2g/m^2/day was given as slow release preparation (Enduramide®) in the 24 hours prior to transplantation. An additional 1g of soluble nicotinamide was given orally immediately prior to transplantation.

$2x10^6$ islet cells (prepared and purified in the presence of 10mM nicotinamide) suspended in saline were then injected intraperitoneally under local anaesthetic after checking the placement of the needle intraperitoneally by x-ray with a small amount of contrast medium.

The number of cells transplanted contained, at most, 10-20 units of insulin, and

therefore, produce a relatively mild insulin reaction, even if all were killed immediately and their insulin released. Hourly blood glucose monitoring and normal means (but without casein) following transplantation, and omission of the insulin injections due at the time transplantation is performed minimised the effect in the unlikely event it occurred. Normal fasting adults can tolerate 10-29 units of quick acting insulin given by subcutaneous injection.

Monitoring of response

1. Insulin requirements/24 hours to maintain near euglycemia.
2. Recurrence of islet cell antibodies.
3. C-peptide measurement of 24 hour procine urinary excretion - at about monthly intervals initially used as an index of the transplant function.

Further testing (oral glucose tolerance) was conducted if insulin requirements disappear.

The nicotinamide and C.M. protein free diet was continued for at least 3 months, and probably indefinitely if insulin requirements disappeared.

Uncontrolled insulin production

It is conceivable that successfully transplanted piglet islets could produce insulin even when blood glucose levels are normal. This has not happened in the piglet to mice experiments, nor in human to human (allograft) experiences internationally.

Piglets islets are killed by the drug streptozotocin, whereas human islets are not. This drug has been used in humans to control inappropriate insulin secretion from malignant islets which are sensitive to the drug.

The likelihood of the above complication is exceedingly remote.

CLINICAL EXAMPLES:

Two such xenotranplants have been carried out in diabetic human subjects.

The first was a 15 year old female who had diabetes for 7 years requiring the injection of daily doses of insulin totalling 76-78 units/day. Despite this her diabetic blood flucose levels were poorly controlled. The Xenotransplant was carried out as above, using 200,000 islets. There was an immediate reduction in insulin requirement which reached its maximum between the 16-21st. day post operatively. During this period average blood glucose control was better than preoperatively. This reduction averaged 18% less than the pretanplant dose during this period. The effect slowly waned

over the next few weeks.

The second transplant involved a 15 year old diabetic male who had the disease for 7 years. On this occasion 800,000 viable islets of more than 150μ in diameter were transplanted. On this occasion the insulin does was reduced to a minimum of 55% of the pretransplant does in the third week post transplant and averages 62% of the pretransplant does in the fifth week after transplantation. The average blood glucose levels before transplantation of about 10mm/l have been reduced to 6.5mm/l in the 4th and 5th weeks. The time course of blood glucose and insulin dose in this subject are shown in Figure 1.

It appears that the transplanted piglet islets are capable of producing insulin for at least 5 weeks after engraftment in diabetic humans and that the magnitude of the effect is related to the number of islets implanted (Figure 2). The duration of the effect in the second instance indicates that acute rejection of the transplanted tissue has not occurred. No side effects of the procedure have been encountered. Further transplant procedures will be carried out using a larger number of islets but in other ways not varying the technique. To date the results in humans are similar to those described in the diabetic mice transplanted with piglet islets.

Although the invention has been described with reference to a number of particular embodiments, it will appreciated by those skilled in the art that the invention may be embodied in many other forms.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method of treatment of a mammalian patient suffering from diabetes which includes the transplantation into the patient of viable porcine islets capable of producing porcine insulin within its host, said islets having been extracted from a piglet at or near full term gestation,

wherein said islets were treated during preparative procedures with nicotinamide and/or any compound exhibiting similar growth promoting and cytoprotective effects,

and wherein said patient, at least for a period after such transplantation, is administered nicotinamide and/or a compound exhibiting similar growth promoting and cytoprotective effects.

2. A method according to claim 1, wherein the mammalian patient is a human patient.

3. A method of claim 1 or claim 2, wherein nicotinamide is used in said preparative procedures.

4. A method of any one of the preceding claims, wherein nicotinamide is administered to said patient.

5. A method of claim 1 wherein 3-amino-benzamide is used in said preparative procedures instead of or in addition to nicotinamide.

6. A method of any one of the preceding claims, wherein 3-amino-benzamide is administered to said patient instead of or in addition to nicotinamide.

7. A method of claim1 or claim 4, wherein nicotinamide is administered orally.

8. A method of any one of the preceding claims further comprising maintainin the patient on a diet low in or devoid of bovine protein.

9. A method of claim 8 further comprising the administration to the patient of a source of protein that substitutes for bovine protein.

10. A method of any one of the preceding claims wherein the treated islets have been subjected to a cryogenic storage period prior to thawing and transplantation.

11. The method of any one of the preceding claims wherein the number of islets transplanted is at least 100,000.

12. A method of any one of the preceding claims wherein the dosage of nicotinamide for transplantation is 1.2-2.4 g/m^2 body surface.

13. A method according to any one of the preceding claims wherein said islets

having been extracted from a piglet delivered naturally.

14. A preparation capable of being injected into a mammalian patient to provide transplantation, including an effective amount of porcine islets capable of producing porcine insulin, said islets having been extracted from a piglet at or near full term gestation, said islets having been treated during preparative procedures with nicotinamide and/or any compound exhibiting similar growth promoting and cytoprotective effects.

15. A preparation of claim 14 in or after having been held in a cryogenically stored form.

16. In an injectable form, a preparation as claimed in claim 14 or 15 having at least 10,000 porcine islets that have been and/or are in a nicotinamide containing environment and which on transplantation are able to multiply.

17. A preparation according to any one of claims 14 to 16, wherein said islets having been extracted from a piglet delivered naturally.

18. A method of treatment of a mammalian patient suffering from diabetes, substantially as herein described with reference to any one of the Examples.

19. A preparation capable of being injected into a mammalian patient to provide transplantation, substantially as herein described with reference to any one of the Examples.

DATED this 25th Day of August, 1998

DIATRANZ LIMITED

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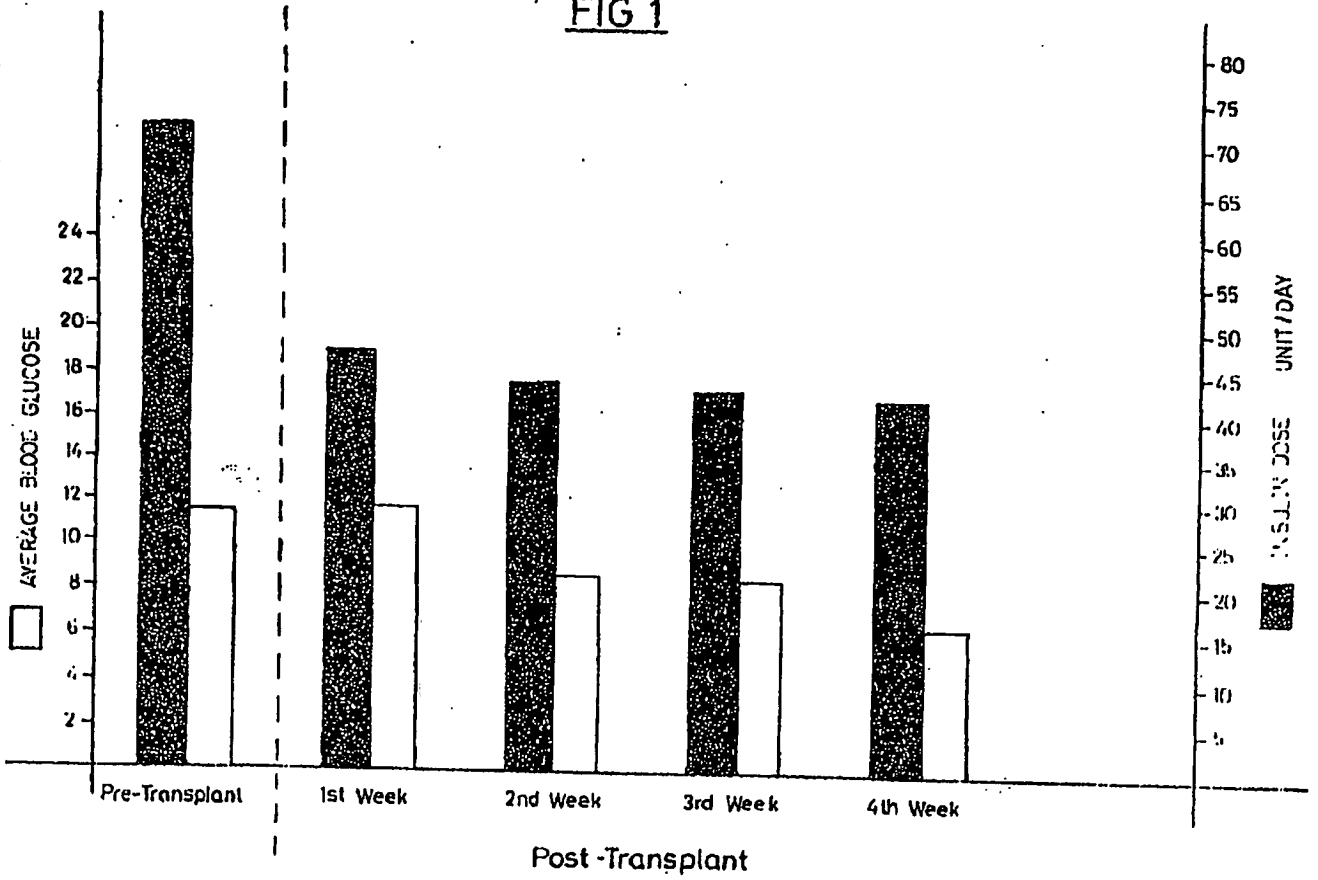
FIG 1



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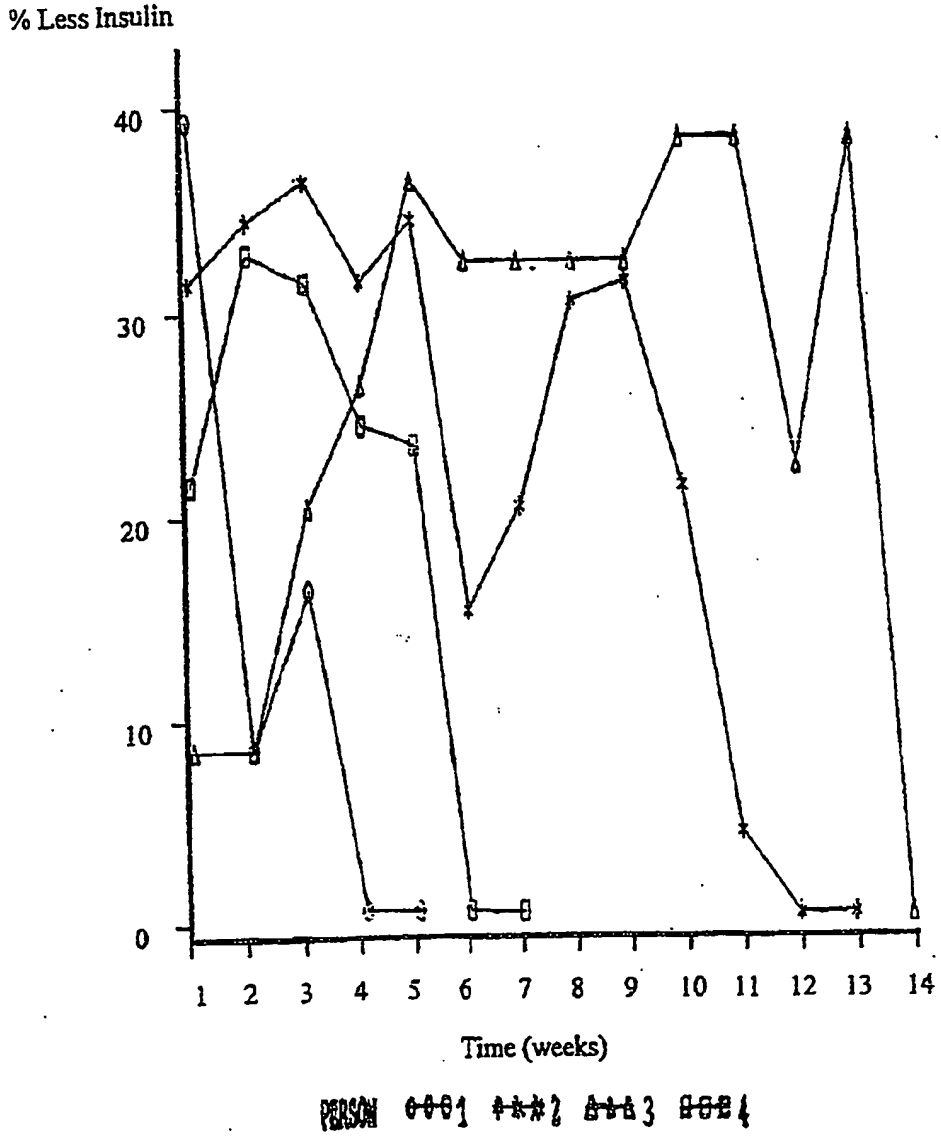

FIGURE 2